Final Report

Microbial Communities of Fire-Affected Soil

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INTRODUCTION

Over 100 years of fire suppression and over grazing by cattle have left profound changes to some of the ponderosa pine forests in Colorado. The forest stand structure has shifted from a wide open savanna-like structure to one with very high tree densities, which in turn has contributed to destructive forest fires such as the Hayman fire of 2002 (Graham, 2003). The current restoration program for the ponderosa pine ecosystem in the Colorado Rocky Mountains involves thinning and reintroduction of fire through prescribed burns to bring back the historical forest structure and function (Dahms and Geils, 1997). Thinning for restoration purposes, in combination with traditional harvesting practices, produces large amounts of slash material, which commonly is disposed of by burning them in large piles. Disposal of slash by piling and burning presents a dilemma to forest land managers. While it is an effective method for removal of unmarketable debris and small trees, soil scorching is detrimental to soil microorganisms (Jiménez-Esquilín et al., 2007), results in unattractive scars, and may assist in the establishment and spread of non-native plants (Korb et al., 2004).

As an alternative to slash piling and burning, thinned forest fuel can be masticated into chips or chunks, which are then spread on the forest floor and left to decompose naturally. Compared to typical harvesting operations, mastication rearranges the amount, size and orientation of surface woody materials. Mastication operations have no natural disturbance analogue; blowdown, fire and insect outbreak all leave woody material intact to decompose rather than converting forest biomass into chip or chunk-size material. Biomass removal and creation of layers of wood residues will likely alter physical,

chemical and biological conditions of the forest floor and upper mineral soil layers, yet the magnitude, duration and implications of such changes are remain largely unknown. A recent review of published findings relating to woody debris additions reported that implementation of chipping and chunking treatments varies considerably among sites depending on equipment and operational differences (Resh et al., 2006). The influence of the treatments on soil properties varied as well, with conflicting reports among the studies. For example, woody debris additions had variable effects on soil nutrients; in some cases, soil N availability increased (Schoenholtz et al., 1992; Wang, 2002), whereas in other studies, N availability was unchanged (Nzila et al., 2002) or decreased as carbon-rich woody material stimulated microbial nitrogen immobilization (Lalande et al., 1998; Binkley et al., 2003; Blumfield and Xu, 2003). Fewer studies have examined mastication treatment effects on soil microbial communities or activities. For example, Waldrop et al. (2003) reported no effects of windrows of chipped forest fuels on enzyme activities in decomposing litter three years after chipping, and Kobziar (2007) found no effect of forest fuels mastication treatment on soil respiration up to two years after the treatment was implemented.

During the summer of 2006, a study was conducted on experimental plots within the Manitou Experimental Forest to investigate the effects forest fuel chips on soil microbial community structure and C and N mineralization activities. Results from this study were reported in the 2007 Annual Report, but in brief, I found that mastication of forest fuels and distribution of these fuels as chips to the forest floor had fewer and less dramatic consequences to soil chemical properties compared to slash pile burning. Two-plus years since chips were added to experimental plots (depths of 0, 5 or 10 cm), there

were statistically significant but small increases in soil EC and concentrations of NH_4-N, Mn, K, and P, depending on the 2006 sampling date. In contrast to a 2006 report by Marchand et al., who analyzed the effects of chipping treatments at a different area of the Manitou Experimental Forest, I did not observe any changes in soil total C in response to chipping treatments. However, chip amendments to the forest floor altered microbial community biomass, structure, and activity. Fungal biovolumes and respiration activity were elevated in 10-cm chipped plot soils throughout most of the 2006 sampling period, whereas bacterial biovolumes but not respiration were elevated in 5- and 10-cm chipped plots at all sampling times. In June, the proportion of active-to-total fungal hyphae increased as chip depth increased. Based on these results, I concluded that chipping treatments have the potential to alter soil fertility, microbial biomass and microbial community structure within the Manitou Experimental Forest.

Here, I report the results of a June 2007 study conducted on the same plots I studied in 2006. This 2007 study examined in greater detail the three-year effects of forest floor chipping amendments on soil fungi, soil carbon, and soil enzyme activities relevant to C substrate decomposition. In addition, a second and larger-scale study was conducted in July 2007 at the experimental location utilized by Marchand et al. (2006) to determine chip amendment effects on soil microbial properties and C. Finally, a third study was conducted in June 2007 to investigate the effects of a lop and scatter mastication treatment on soil microbial properties and C. The objectives of the three studies were to determine soil ecosystem consequences of various mastication treatments, applied at different scales within the same ponderosa pine forest, with an emphasis on soil microbial community structure, function and soil carbon (C).

MATERIALS AND METHODS

Study Descriptions

All studies were conducted at the Manitou Experimental Forest (MEF), centrally located in the Rocky Mountains (39° 04' North and 105° 04' West) approximately 45 km west of Colorado Springs, Colorado. The mean annual temperature of the forest is 5°C, the mean annual precipitation is 40 cm, and its mean elevation is 2400 m. The study area is occupied by an overstory of mature (>150 y of age) ponderosa pine (*Pinus ponderosa* P. & C. Lawson) and Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco), and an understory of artemesia, bunchgrasses, and other forbs and grasses. The soils at MEF originated from gravelly alluvium and outwash of Pikes Peak granite and are classified as loamy mixed Eutroboralfs or Aridic Haploborolls (Moore, 1992). Soils within this particular area are approximately 66% sand, 21% silt, and 13% clay with bulk densities that usually increase with depth and range between 1.1 and 1.5 g cm^{-3}. Soil organic material comprises about 1–2% of the soil by volume. Previous grazing and mechanical harvesting throughout the area has resulted in a moderately disturbed soil. More details about MEF can be found in Massman and Frank (2004) and Shepperd et al. (2006).

Plot-level chipping study

Two experimental blocks were located fairly close to each other (< 0.5 km), with one block located under a forest canopy and the second block in an open meadow, with no trees. Each block contained three experimental plots of approximately 20 to 50 km in length. In March 2004, one plot of each block received 5 cm of chipped ponderosa pine forest fuels, one plot received 10 cm of chips, and one plot received none (control).

Larger-scale chipping study

A study was initiated in 2004 on a ~12 ha area of the MEF which included three treatments, imposed on approximately the same-size areas: nontreated control, whole-tree harvesting with all chipped biomass retained on site (thinned + chips treatment) and whole-tree harvesting with all thinned material removed (thinned treatment). For the thinned treatment, trees were felled and skidded intact to a landing site outside the treatment area, with a minimum of logging debris left on site (Marchand et al., 2006). Thinning operations reduced basal area from 25.5 m^2 ha^{-1} to ~ 11.5 m^2 ha^{-1}. For the thinned + chips treatment, a whole-tree harvester with a chipper was used to fell and chip trees in place. The average depth of chips on the forest floor of the thinned + chips area was 2 cm.

Lop and scatter plot-level study

Similar to the plot-level chip study, the study design of the lop and scatter experiment consisted of two experimental blocks, each with two plots approximately 50 m in length. In 2004, ponderosa pine slash material was lopped and scattered on the forest floor (~ 47 Mt ha^{-1} loading rate) of one plot per block, with the second plot receiving no treatment.

Soil Sampling

Plot-level chipping and lop and scatter studies

In June, 2007, composite soil samples were collected (0-10 cm) from each plot of the chip and lop-and-scatter experimental blocks. Each composite sample consisted of three-to-five soil samples collected with a hand trowel. For the lop and scatter study, soil samples were collected from areas directly under the woody debris, as well as from open spaces between the woody debris. These samples were analyzed separately, to create three lop and scatter "treatments": control, under debris of lop and scatter, and under no debris of lop and scatter. In all, there were six soil samples, or experimental units, for the chip plot study, and six soil samples (experimental units) for the lop and scatter study.

All soil samples were stored in Ziploc freezer bags and transported on ice to the laboratory. Soils were passed through a 2-mm mesh sieve and stored at 4°C for determination of fungal biomass, C and N mineralization activity, and β-glucosidase and acid phosphatase enzyme activities; at -80°C for peroxidase enzyme activity; and air-dried for soil C and total N determinations.

Larger-scale chipping study

In July 2007, composite soil samples (0-10 cm depth) were collected with a trowel every 50 m along a 500-m transect in each treated and non-treated area. Each composite sample consisted of three-to-five soil samples. There were a total of ten soil samples, or experimental units, per treatment (control, thinned + chips, and thinned). All soil samples were stored in Ziploc freezer bags and transported on ice to the laboratory. Soils were passed through a 2-mm mesh sieve and stored at 4°C for determination of

fungal biomass, C and N mineralization activity, and β-glucosidase and acid phosphatase enzyme activities; at -80°C for peroxidase enzyme activity; and air-dried for soil C and total N determinations.

Soil Analyses

Fungal biomass

Fungal biovolumes in soil were determined by direct microscopy. For the chip and lop-and-scatter plot studies, each experimental unit was analyzed in duplicate analytical replicates. One subsample was analyzed per experimental unit of the larger-scale chipping study. Subsamples (10 g dry weight) of soil were serially diluted (100-fold) in filter-sterilized water, and soil fungal hyphal lengths were quantified by the coverslip-well slide method of Lodge and Ingham (1991). Fungal slides were observed at 400 × resolution with a Nikon Eclipse E600 epifluorescence microscope (Nikon Instruments, Inc., Mellville, NY) equipped with an ocular grid. For each soil sample, fungi were counted in a total of 30 fields of view. Images of fungal hyphae were captured with a CoolSNAP Pro_{cf} digital camera (A.G. Heinze Precision MicroOptics, Lake Forest, CA) and ImagePro Plus imaging software (Media Cybernetics, Silver Spring, MD). Biovolume conversions of fungi were determined based on the average diameter of at least 80 hyphal fragments (Klein and Paschke, 2000). Fungal biomass C was estimated by multiplying biovolumes by 0.33 g cm^{-3} and assuming 40% C (van Veen and Paul, 1979; Frey et al., 1999).

Potential C and N mineralization activities

For the chip and lop-and-scatter plot studies, each experimental unit was analyzed in duplicate analytical replicates. One subsample was analyzed per experimental unit of the larger-scale chipping study. Soil C mineralization activity was determined during a 28-day aerobic incubation which employed an alkali-trap method (Zibilske, 1994). Each soil subsample (10.0 g dry weight) was placed in a sealed 0.95-L airtight glass Mason jar. Soils were adjusted to 75% water holding capacity by adding distilled water. A vial containing 12.5 mL of 1.0 N NaOH was added to each jar, and the jars were maintained in the dark at 25°C for 28 days. At day 28 of the incubation, vials of NaOH were removed, and the CO_2 respired was measured by titration with 0.773 N HCl to a phenolphthalein endpoint. Duplicate blank jars without soil were included to determine background CO_2 and all samples were corrected accordingly.

Net N mineralization (NO_3-N + NH_4-N) was determined by extracting 10 g of freshly sampled and day 28 soils from the above incubation in 50 ml of 2 M KCl on a shaker (180 rpm) for one hour (Mulvaney, 1996). Each sample was filtered through Whatman #1 filter paper and analyzed for NO_3^- and NH_4^+ on a Perstorp Enviroflow flow injector (Perstorp Analystical, Inc., Silver Spring, MD). Net N mineralized was determined by subtracting the NO_3-N + NH_4-N concentration at the beginning of the incubation period from the concentration at the end of the period for each sample.

Soil enzyme activities

Enzyme assays were performed according to the methods described by Sinsabaugh et al. (2003). β-glucosidase and acid phosphatase were determined

fluorimetrically, whereas peroxidase was determined colorimetrically. β-glucosidase is an enzyme involved in carbohydrate decomposition, and specifically catalyzes the hydrolysis of maltose or cellobiose into glucose monomers. Acid phosphatase hydrolyzes phosphate from phosphomonoester compounds (including nucleotides and phospholipids), and peroxidase catalyzes lignin hydrolysis. A 1.0 g subsample of each soil sample was suspended in 125 mL of 50 mM, pH 5.0 acetate buffer. The buffer was homogenized at high speed for 1 minute on a Waring blender. For β-glucosidase and acid phosphatase assays, the resulting suspensions were continuously stirred using a magnetic stir plate as 200 μL aliquots were distributed into 96-well microtiter plates. Because of colorimetric interference from soil particles, the peroxidase assay was conducted with supernatant collected after centrifuging soil suspensions at 2500 rpm for 10 minutes.

For the fluorimetric assays, 50 μL of 200 μM substrate solution (4-methylumbelliferone-β-D-glucoside or 4-methylumbelliferone-phosphate) were added to sample wells. Blank wells received 50 μl of acetate buffer and 200 μL of sample suspension. Negative control wells received 50 μL substrate solution plus 200 μL acetate buffer. Quench standard wells received 50 μL of 10 μM methylumbelliferone standard and 200 μL of sample suspension. Reference standard wells received 50 μL of standard and 200 μL acetate buffer. There were 16 replicate wells for each sample and eight replicate wells for each blank, negative control, quench standard, and reference standard. Microplates were incubated in the dark at 20°C for 4 hours. After the incubation, 10 μL of 1.0 M NaOH was added to each well to stop the reaction. Fluorescence was measured with a microplate fluorometer with 365 nm excitation and 450 nm emission filters.

Samples were corrected for blank, control, and quenching activities, and sample activity was expressed in units of μmol g^{-1} soil h^{-1} or μmol g^{-1} total soil organic C h^{-1}.

The peroxidase assay utilized L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate. Sample wells contained 50 μL of 25 mM L-DOPA plus 25 μL of 0.12% H_2O_2. Negative control wells contained 200 μL acetate buffer, 50 μL L-DOPA, and 25 μL 0.12% H_2O_2. Blank wells contained 200 μL of soil suspension, 50 μL acetate buffer, and 25 μL 0.12% H_2O_2. There were 16 replicate wells for each sample and eight replicate wells for each blank and negative control. Microplates were incubated in the dark at 20°C for 4 hours. Absorbance was read at 450 nm with a microplate spectrophotometer, and after correcting sample absorbances for the blank and control, activity was expressed in units of nmol g^{-1} soil h^{-1}.

Soil organic C and total N

A method modified from Sherrod et al. (2005) was employed to quantify the concentration of soil C distributed among three soil pools: total organic C (TOC), particulate organic matter C (POM-C), and mineral-associated organic C (MAOC). A 10-g subsample of air-dried soil, sieved to pass a 2-mm mesh screen and with all visible plant material larger than 2 mm removed, was suspended in 40 mL of sodium hexametaphosphate (5 g L^{-1}) and shaken overnight at 180 rpm. The soil suspension was poured over a 53 μm-mesh sieve, and the material retained on the top of the sieve was designated as the POM fraction. The POM fraction was gently rinsed in distilled water until the water draining from the sieve was clear. The POM fraction was quantitatively transferred with distilled water to a stainless steel bowl of known tare weight, and bowls

were placed in a 50°C oven to dry overnight. The dry POM fraction was then weighed to determine the weighted fraction of POM to whole soil. The POM fraction and a subsample of whole soil were powder-ground with a ball mill grinder to pass an 80-μm sieve. Samples were analyzed for total C on a Leco-CHN-1000 auto analyzer (Leco, St. Joseph, MI) and inorganic C by the pressure calcimeter method (Sherrod et al., 2002). Inorganic C concentration was negligible, so C measured on the Leco was considered to be organic C. The POM fraction C concentration was converted to a per kg soil basis, and MAOC was calculated as the difference between whole soil TOC and POM-C.

Statistical Analyses

Statistical analyses were performed using the SAS statistical Package version 9.1 (SAS Institute Inc, Cary, NC). The experimental design of the plot-level chipping study and the lop-and-scatter study was a randomized complete block design with two statistical replicates/blocks. Analysis of variance was conducted on all univariate data with treatment (chipping depth or lop-and-scatter treatment) as the class variable (α=0.10). Fishers' protected least significant difference (LSD) values were calculated to separate means when the ANOVA showed significance ($p < 0.10$).

For the larger-scale chipping study, data were analyzed by multi-response permutation procedure (MRPP), which is a nonparametric procedure for testing the hypothesis of no difference between two or more groups of entities (McCune and Grace 2002). I used a MRPP macro developed for Microsoft Excel by Rudy King, USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO (rmking@fs.fed.us). The distance measure employed for this particular MRPP is Euclidean distance. For each

variable, the null hypothesis of no difference among treatments was rejected when $p < 0.10$. When significant differences were found, multiple comparisons among groups were computed based on the Peritz closure method (Petrondas and Gabriel, 1983).

RESULTS AND DISCUSSION

Chipping Studies

Fungal biomass C was significantly greater in soil underlying 10 cm of ponderosa pine chips (Fig. 1), with more than twice the amount of biomass compared to non-treated plot soil. However, despite the change in fungal biomass, microbial and soil enzyme activities were not significantly different among the treatment plots. There were trends for less C and N mineralization activities, less β-glucosidase and acid phosphatase activities, but greater peroxidase activity, in soil under 10 cm of chips compared to non-treated soil, but these trends were not statistically significant (Table 1). Because microbial activities were not significantly affected by chipping treatments, it was not surprising to find that there were no differences in soil TOC, POM-C, MAOC, soil TN, or soil C:N ratio among the treatment plots (Table 2).

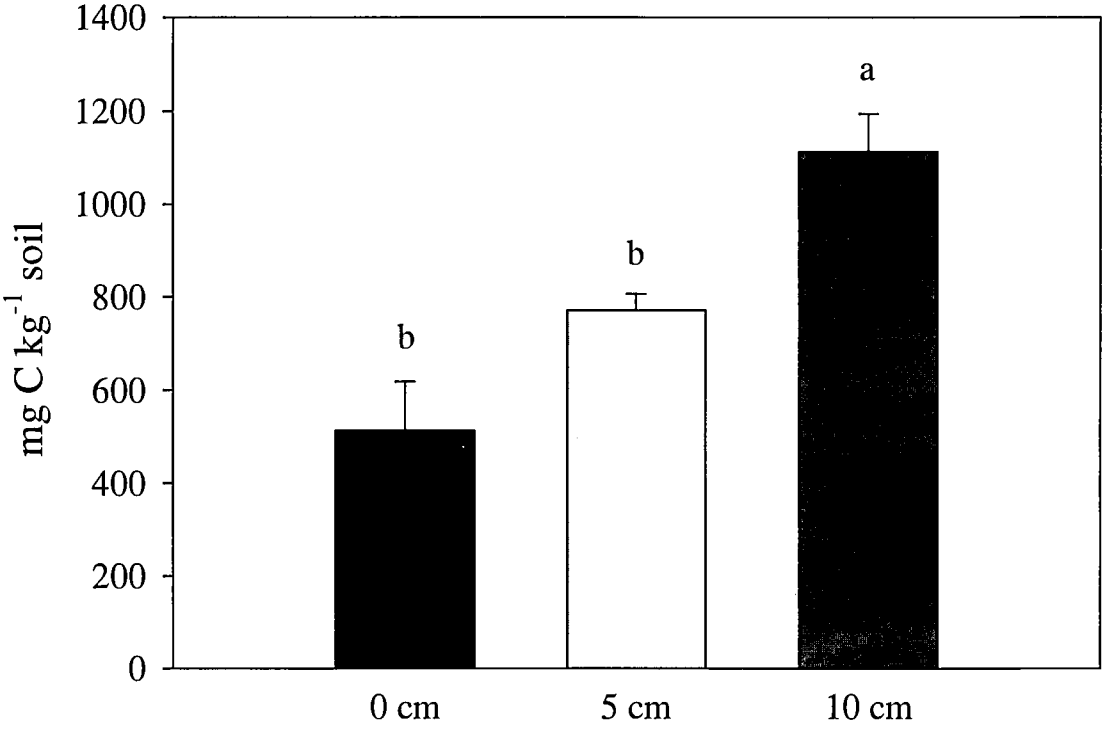

Fig. 1. Fungal biomass C of a ponderosa pine forest soil (0-10 cm depth) underlying wood chips that were added to the forest floor at different depths in March 2004. Data are from soils collected in June 2007. Histogram bars labeled with different lowercase letters are significantly different at $\alpha = 0.10$. Error bars are one standard error of the mean (n=2).

Table 1. Microbial mineralization and soil enzyme activities of a ponderosa pine forest soil (0-10 cm depth) underlying wood chips that were added to the forest floor at different depths in March 2004. Data are from soils collected in June 2007. Column means followed by different letters are significantly different at $\alpha = 0.10$. Standard errors (± 1) are shown in parentheses (n=2).

Chip depth (cm)	C mineralization	N mineralization	β-glucosidase	Acid phosphatase	Peroxidase
	mg CO_2-C kg^{-1} soil	mg N kg^{-1} soil	μmole product g^{-1} soil h^{-1}		nmol product g^{-1} soil h^{-1}
0	18.9 (6.9)	0.160 (0.001)	175 (34)	262 (83)	403 (119)
5	8.92 (6.99)	0.131 (0.022)	149 (72)	312 (90)	447 (151)
10	8.96 (1.65)	0.092 (0.024)	113 (23)	214 (9)	659 (430)

Table 2. Concentrations of organic C, total N, and C:N ratios, of a ponderosa pine forest soil (0-10 cm depth) underlying wood chips that were added to the forest floor at different depths in March 2004. Data are from soils collected in June 2007. Column means followed by different letters are significantly different at $\alpha = 0.10$. Standard errors (± 1) are shown in parentheses (n=2).

Chip depth (cm)	Total organic C	Particulate organic matter C	Mineral-associated organic C	Total N	C:N ratio
	---------- g kg^{-1} soil ----------				
0	22.9 (3.7)	7.36 (0.14)	15.5 (3.6)	1.54 (0.45)	15.5 (2.1)
5	18.8 (2.0)	6.19 (1.86)	12.6 (0.2)	1.19 (0.12)	16.2 (3.3)
10	21.1 (1.5)	6.37 (0.81)	14.7 (0.7)	1.35 (0.06)	15.7 (1.8)

More treatment differences were observed within the larger-scale chipping study. Soils from the thinned-only treated area contained significantly less (35 to 45% less) fungal biomass C than non-treated soil and soil from the thinned + chips area (Fig. 2). While thinned + plus chips treatment did not result in greater fungal biomass compared to the control treatment (in contrast to the plot-level study), the addition of chips to the forest floor apparently prevented a reduction in soil fungal biomass, which occurred in response to forest thinning. Interestingly, microbial C and N mineralization activities were greatest in the thinned treatment soil (Fig. 3), indicating that soil with less fungal biomass has less potential to immobilize C and inorganic N compared to soil with greater fungal biomass (control and thinned + chips soils).

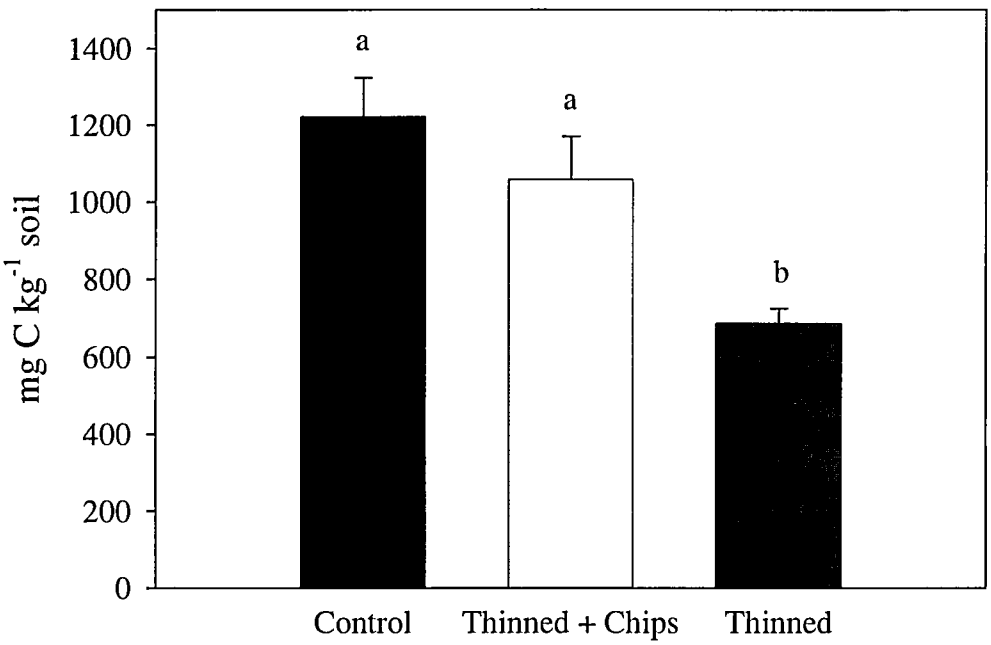

Fig. 2. Fungal biomass C of a ponderosa pine forest soil (0-10 cm depth) collected in July 2007 from transects within a non-treated area (control), a thinned area with chipped slash added to soil surface (thinned + chips), and thinned area with no chip addition (thinned). Histogram bars labeled with different lowercase letters are significantly different at α = 0.10. Error bars are one standard error of the mean (n=10).

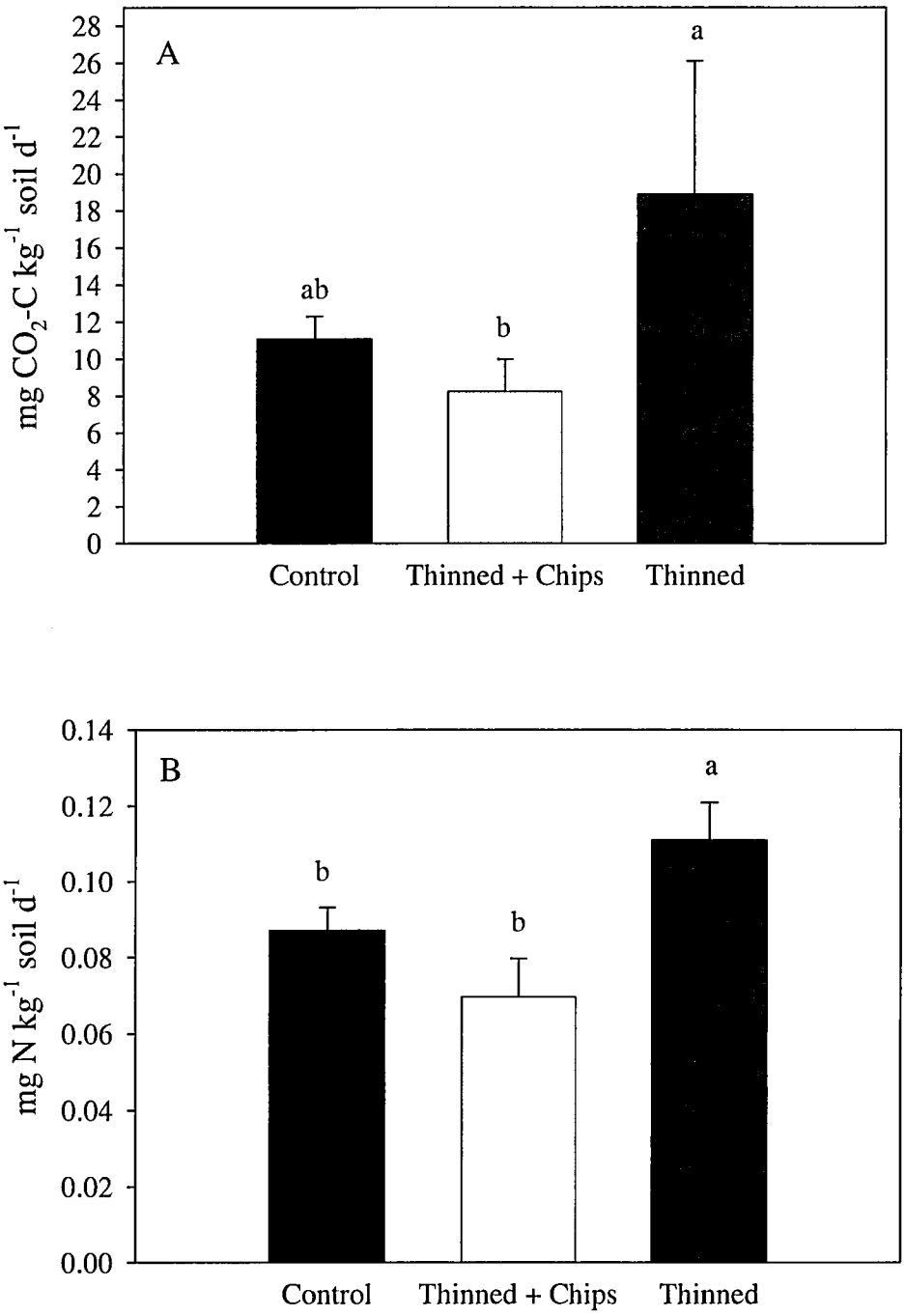

Fig. 3. Carbon and N mineralization activities of a ponderosa pine forest soil (0-10 cm depth) collected in July 2007 from transects within a non-treated area (control), a thinned area with chipped slash added to soil surface (thinned + chips), and thinned area with no chip addition (thinned). Histogram bars labeled with different lowercase letters are significantly different at $\alpha = 0.10$. Error bars are one standard error of the mean (n=10).

When soil enzyme activities were expressed on a per g soil basis, there were no significant differences in activities among the non-treated, thinned, and thinned + chips soils (Table 3), although there was a trend for peroxidase activity to be reduced in the thinned area. This trend for peroxidase activity was expected, because trees (and therefore a source of C material) were removed from this area and lignin-rich chips were not added to the forest floor. However, when enzymes were expressed on a per g TOC basis, as a means of standardizing enzyme activities across the study area, there was a significant difference in acid phosphatase activity among the treatment areas (Fig. 4). Specifically, acid phosphatase activity was ~ 30% greater in the thinned + chips soil compared to the other soils. This enzyme was assayed because in the 2006 study, significantly greater concentrations of extractable soil P were detected in chipped plots of the plot-level chipping study. This led me to hypothesize that chip amendments to the forest floor increase the production of phosphatase enzymes so that P would be less limiting to soil microorganisms as chip C is degraded. This could be the phenomenon occurring in the larger-scale chipping study site. However, increased acid phosphatase activity is not a universal response to chipping as this did not occur in the plot-level chipping study site, even when acid phosphatase was expressed on a per g TOC basis.

Table 3. Enzyme activities of a ponderosa pine forest soil (0-10 cm depth) collected in July 2007 from transects within a non-treated area (control), a thinned area with chipped slash added to soil surface (thinned + chips), and thinned area with no chip addition (thinned). Column means followed by different letters are significantly different at α = 0.10. Standard errors (± 1) are shown in parentheses (n=10).

Treatment	β-glucosidase	Acid phosphatase	Peroxidase
	μmole product g^{-1} soil h^{-1}		nmol product g^{-1} soil h^{-1}
Control	214 (12)	198 (16)	1033 (135)
Thinned + chipped	180 (22)	184 (21)	1119 (151)
Thinned	197 (24)	185 (24)	855 (204)

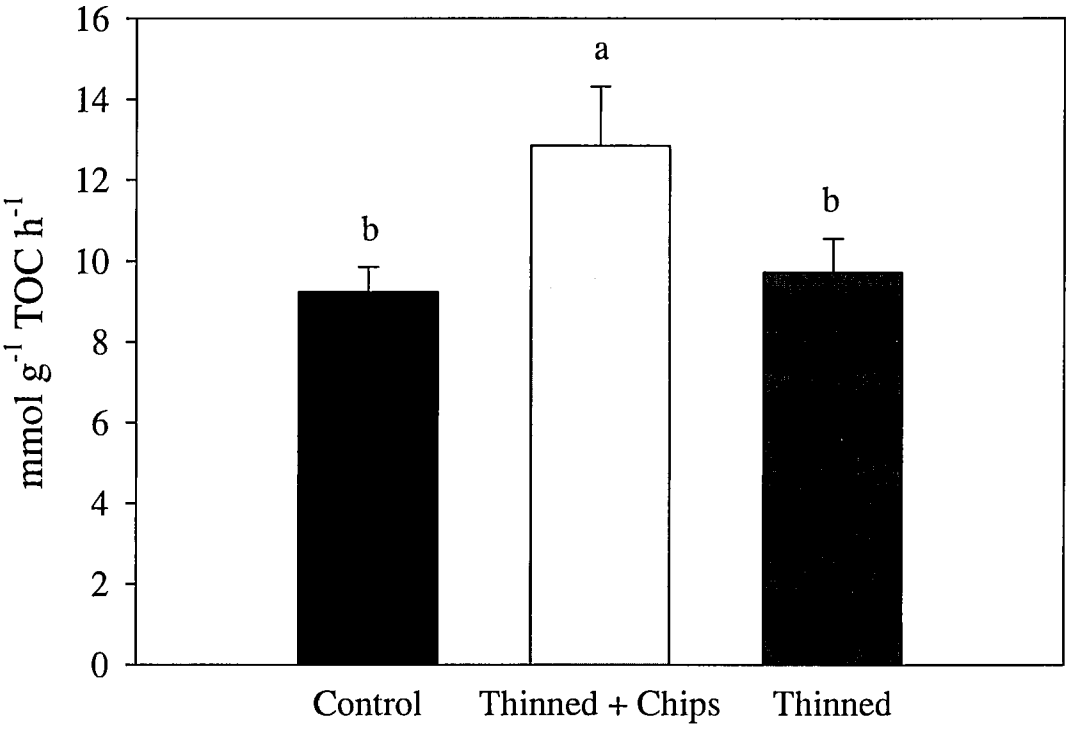

Fig. 4. Acid phosphatase enzyme activity, expressed on a per g total organic C basis, of a ponderosa pine forest soil (0-10 cm depth) collected in July 2007 from transects within a non-treated area (control), a thinned area with chipped slash added to soil surface (thinned + chips), and thinned area with no chip addition (thinned). Histogram bars labeled with different lowercase letters are significantly different at α = 0.10. Error bars are one standard error of the mean (n=10).

Through their aggregation formation and stabilization processes, fungi play an important role in sequestering C in soil (Six et al., 2006). In the present study, soil fungal biomass was less and C mineralization activity was greater in the thinned area relative to non-treated and thinned + chips soils. However, there was no evidence that soil organic C concentrations were detrimentally affected by thinning as soil TOC, POM-C, and MAOC concentrations did not differ significantly between non-treated and thinned area soils (Fig. 5). Instead, TOC was significantly less in soil from the thinned + chips area (32% less compared to thinned soil, and 40% less compared to non-treated soil) , and reduction in TOC was associated with reductions in both POM-C and MAOC (Fig. 5). This was an unexpected finding because fungal biomass C and C cycling activities (C mineralization potential, β-glucosidase and peroxidase enzyme activities) were not significantly affected by thinning and chipping compared to no treatment. However, this finding is consistent with that of Marchand et al. (2006), who also measured significantly less TOC in thinned + chips soil compared to soil from the non-treated area. Marchand et al. (2006) speculated that the reduction in TOC in thinned + chips soil could be due to the lack of significant movement of C from the forest floor into soil combined with considerable soil microbial respiration.

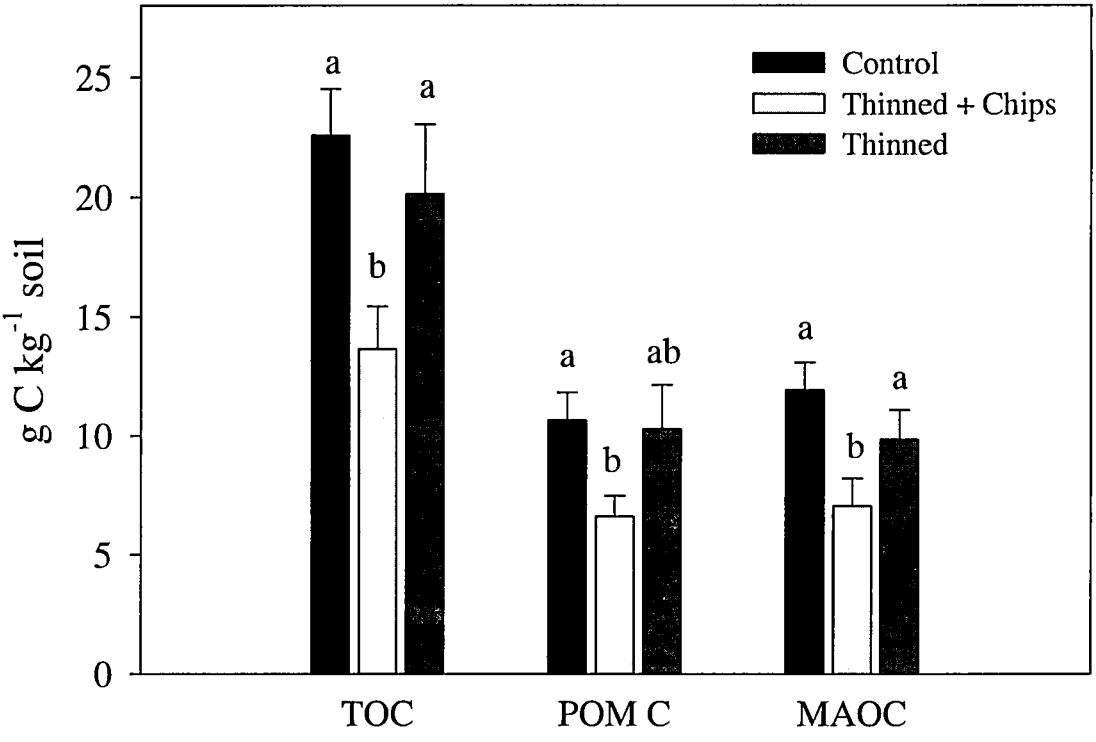

Fig. 5. Concentrations of total organic C (TOC), particulate organic matter C (POM C) and mineral-associated organic C (MAOC) in a ponderosa pine forest soil (0-10 cm depth) collected in July 2007 from transects within a non-treated area (control), a thinned area with chipped slash added to soil surface (thinned + chips), and thinned area with no chip addition (thinned). Within a C fraction, histogram bars labeled with different lowercase letters are significantly different at $\alpha = 0.10$. Error bars are one standard error of the mean (n=10).

Another possible explanation is that the initial input of labile dissolved organic matter (DOM) from chips into soil stimulated the soil microbial community and contributed to the degradation of soil organic matter, a concept known as "priming" (Bingemann et al., 1953). This priming effect is likely due to increased extracellular enzyme production and microbial biomass fueled by the addition of labile DOM (Shen and Bartha, 1996; Hamer and Marschner, 2002; Fontaine et al., 2004). In the present study, we did observe that thinned trees retained on the forest floor as chips resulted in an increase in fungal biomass and acid phosphatase activity relative to the thinned-only treatment. A priming effect would also explain why total N concentration was significantly less by 25% in thinned + chips soil compared to non-treated soil (Fig. 6), as degradation of soil organic matter would result in decreased soil N.

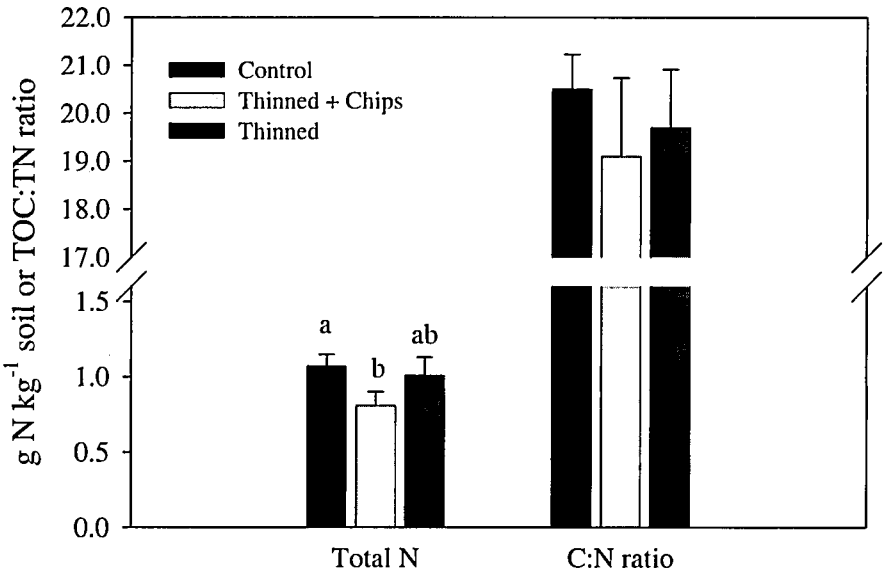

Fig. 6. Total N concentration and ratio of total organic C-to-total N of a ponderosa pine forest soil (0-10 cm depth) collected in July 2007 from transects within a non-treated area (control), a thinned area with chipped slash added to soil surface (thinned + chips), and thinned area with no chip addition (thinned). Within each soil property, histogram bars labeled with different lowercase letters are significantly different at $\alpha = 0.10$. Error bars are one standard error of the mean (n=10).

Recently, Carney et al. (2007) examined the effect of elevated soil CO_2 levels on microbial community structure and function in a scrub-oak ecosystem. They found that soil exposed to elevated CO_2 had less organic C than soil exposed to ambient CO_2, and they attributed this reduction to more rapid rates of soil organic matter decomposition because of greater relative abundances of fungi and greater phenol oxidase activity in soils exposed to elevated CO_2. While I did not measure soil CO_2 levels in the larger-scale field study, Massman et al. (2006) measured significantly greater concentrations of CO_2 in soil underlying chips of the plot-level chipping study. This is likely a consequence of the chips acting as a barrier to the normal CO_2 efflux from the soil (Massman et al., 2006). Therefore, it is possible that soil CO_2 may have been elevated under the chips of the thinned + chips treatment, which could have resulted in an increase in soil fungal biomass and therefore greater decomposition rates relative to the thinned-only treatment. The weakness of this explanation, however, is that I did not find reduced organic C levels in soil underlying 5 or 10 cm of chips of the plot-level study, even though these treatments significantly increased soil fungal biomass and soil CO_2 levels.

Lop and Scatter Study

The two plot-level studies were established and sampled at the same time and allowed for a comparison between contrasting mastication treatments, chipping versus lop and scatter. In contrast to the plot-level chipping study, fungal biomass C was not significantly affected by the lop and scatter treatment (Fig. 7). Also in contrast to the chipping study, microbial potential N mineralization activity was significantly impacted by the lop and scatter treatment, with 33% less mineralization activity in treatment plot

soils than in non-treated plot soil (Fig. 8). Similar to the chipping study, the lop and scatter treatment had no effect on microbial C mineralization activity (Fig. 8) or soil enzyme activities (Table 4), although there was a trend for peroxidase enzyme activity to be greater in soil from the lop and scatter plots than in soil from the non-treated plots. Concentrations of soil organic C (Table 5) and total N (Fig. 9) were not significantly impacted by the lop and scatter treatment, but soil C:N ratio was significantly greater directly under the woody debris (13.3) compared to the other soils (12.8 in soils not directly under debris, and 12.5 in non-treated soil) (Fig. 9).

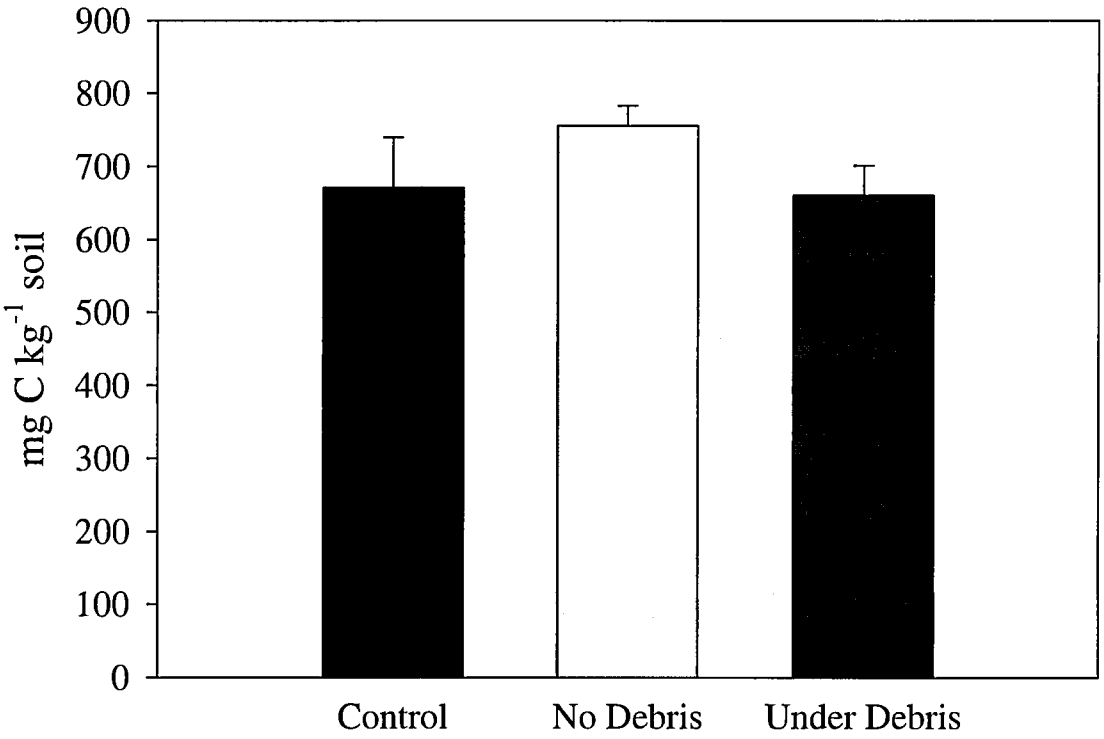

Fig. 7. Fungal biomass C of a ponderosa pine forest soil subjected to a lop-and-scatter (LS) mastication treatment in March 2004. Soil samples (0-10 cm depth) were collected in June 2007 from non-treated plots (control), under no debris within LS plots (no debris), and directly under debris within LS plots (under debris). Error bars are one standard error of the mean (n=2).

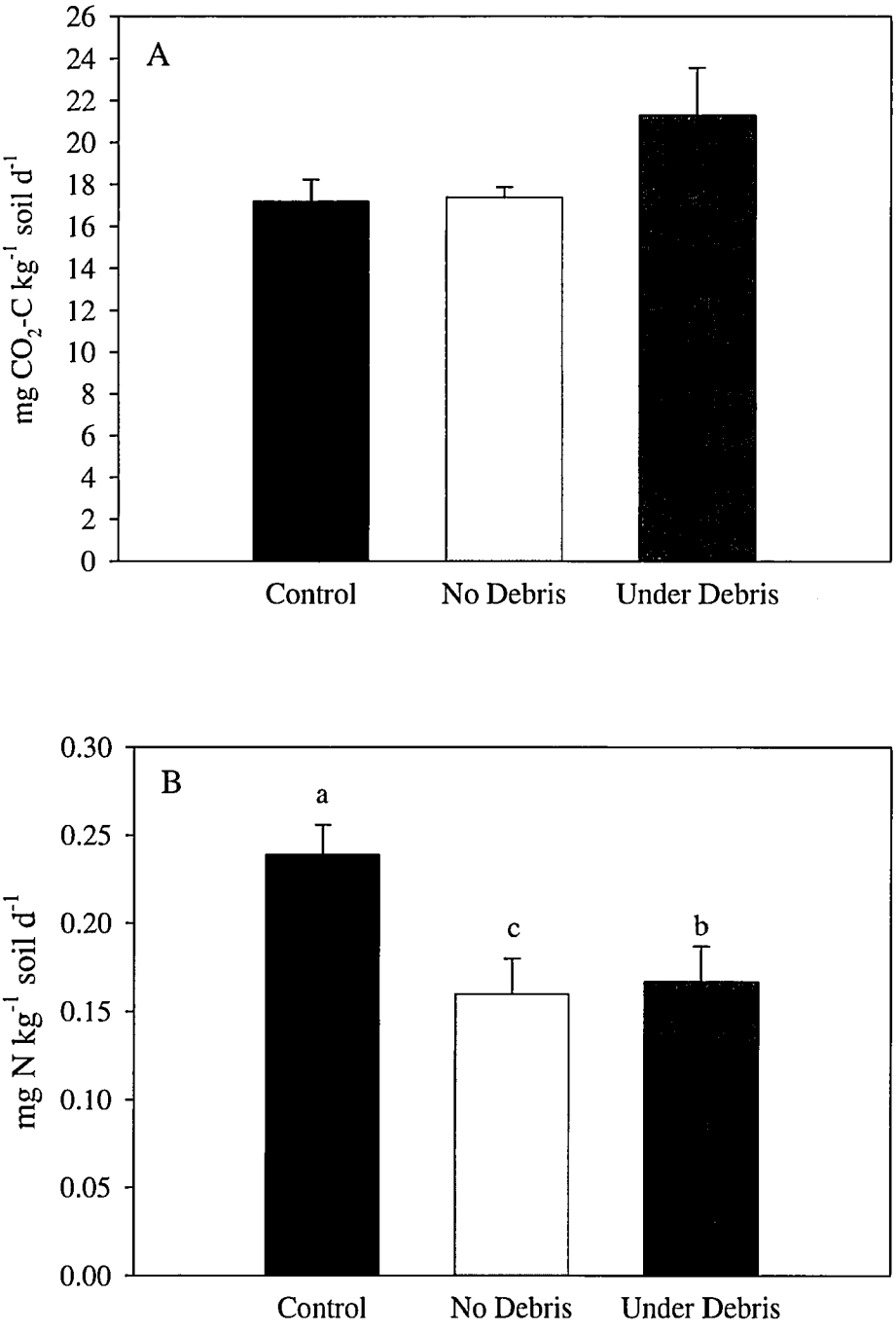

Fig. 8. Carbon and N mineralization activities of a ponderosa pine forest soil subjected to a lop-and-scatter (LS) mastication treatment in March 2004. Soil samples (0-10 cm depth) were collected in June 2007 from non-treated plots (control), under no debris within LS plots (no debris), and directly under debris within LS plots (under debris). Histogram bars labeled with different lowercase letters are significantly different at α = 0.10. Error bars are one standard error of the mean (n=2).

Table 4. Enzyme activities of a ponderosa pine forest soil (0-10 cm depth) subjected to a lop-and-scatter (LS) mastication treatment in March 2004. Data were collected in June 2007. Column means followed by different letters are significantly different at $\alpha = 0.10$. Standard errors (± 1) are shown in parentheses (n=2).

Treatment	β-glucosidase	Acid phosphatase	Peroxidase
	μmole product g^{-1} soil h^{-1}		nmol product g^{-1} soil h^{-1}
Control (no treatment)	158 (18)	247 (26)	260 (13)
LS, soil under no debris	176 (15)	256 (40)	320 (33)
LS, soil directly under debris	164 (9)	253 (4)	381 (160)

Table 5. Concentrations of organic C from a ponderosa pine forest soil (0-10 cm depth) subjected to a lop-and-scatter (LS) mastication treatment in March 2004. Data were collected in June 2007. Column means followed by different letters are significantly different at $\alpha = 0.10$. Standard errors (± 1) are shown in parentheses (n=2).

Treatment	Total organic C	Particulate organic matter C	Mineral-associated organic C
	----------	g kg^{-1} soil ----------	
Control (no treatment)	23.2 (4.0)	7.38 (0.99)	15.8 (3.0)
LS, soil under no debris	25.2 (4.5)	8.15 (1.91)	17.0 (2.6)
LS, soil directly under debris	24.5 (2.5)	7.57 (0.66)	16.9 (1.8)

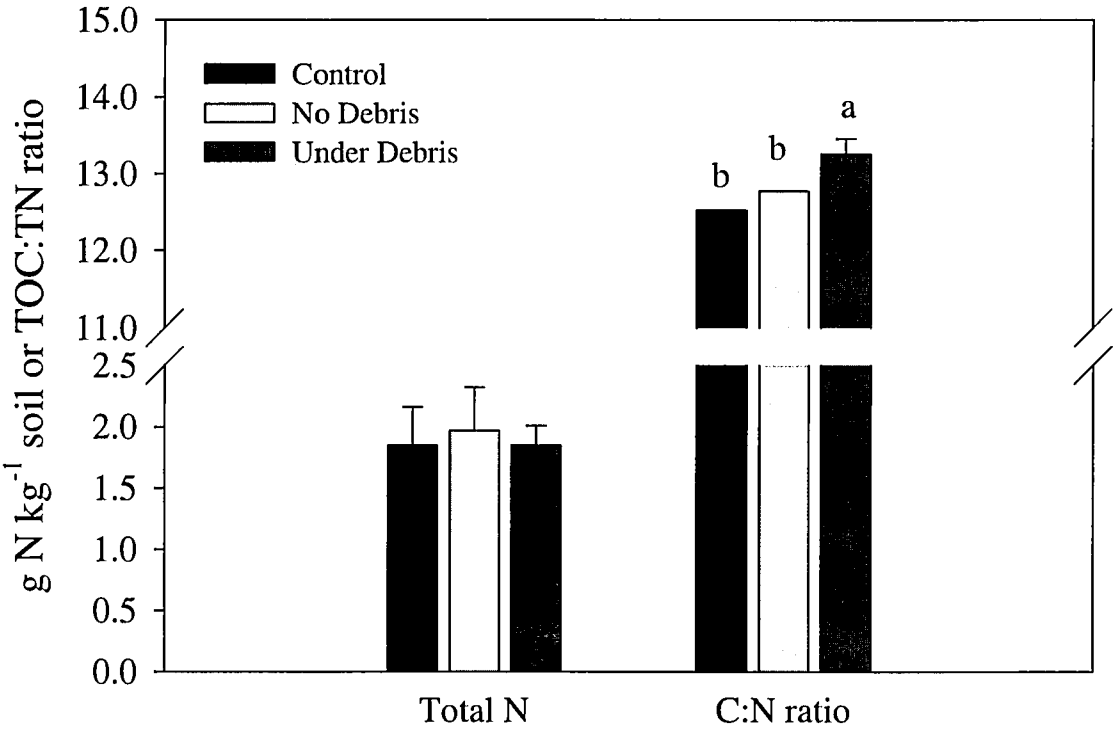

Fig. 9. Total N concentration and ratio of total organic C-to-total N of a ponderosa pine forest soil subjected to a lop-and-scatter (LS) mastication treatment in March 2004. Soil samples (0-10 cm depth) were collected in June 2007 from non-treated plots (control), under no debris within LS plots (no debris), and directly under debris within LS plots (under debris). Histogram bars labeled with different lowercase letters are significantly different at $\alpha = 0.10$. Error bars are one standard error of the mean (n=2).

SUMMARY AND CONCLUSIONS

The plot-level chipping study demonstrated that a relatively thick layer of chips added to the forest floor (10 cm) can increase soil fungal biomass. However, three years after the treatment was implemented, there was no effect of the chips on microbial and soil enzyme activities we measured, nor on soil organic C and total N levels. The larger-scale study demonstrated that thinning practices can negatively impact soil fungal biomass and increase the mobilization of C and N through increased microbial respiration and N mineralization activities. The reduction of soil fungal biomass can be prevented, however, if thinned material is chipped and applied to the forest floor. However, this study also found that a relatively thin layer of chips added to the forest floor can significantly reduce soil organic C levels. It is possible that in the present study, this thin layer of chips added just enough soluble C to the soil to stimulate microbial degradation activity, and when this labile source of C was depleted, microorganisms turned to soil organic matter as a source of C. It is not clear, however, why chips added to a depth of 5 or 10 cm in the plot-level study did not cause a similar priming effect and reduction of soil organic carbon. Perhaps these thicker layers of chips are still contributing some labile DOM to the soil microbial community, and therefore, priming of soil microorganisms to degrade soil organic matter has not yet occurred in these plots.

I also found that a lop and scatter mastication treatment differently affected soil properties compared to chipping treatments, mainly by reducing N mineralization potential and increasing the C:N ratio of the soil. The immobilization of soil N, as well as the slight increase in peroxidase activity directly under the woody debris, is indicative of DOM leaching from the woody debris into soil. In conclusion, I found that forest fuels

management practices which differ in terms of mastication method and dispersal depth have different effects on soil properties at a given sampling time. Perhaps the treatments studied will all affect soil microorganisms the same way, but at different times depending on the size of masticated material and depth/loading rate at which the material is applied. Future studies should focus on mechanisms by which soil properties are affected by mastication treatments, including quantity and rates of C inputs from masticated materials. Such mechanistic-based information would provide managers with a better ability to predict soil responses to forest fuels reduction practices.

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